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Increased Sensitivity of PCR Detection of *Staphylococcus aureus* and *Vibrio cholerae* Using a Non-specific Amplification Step

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We have developed a method to increase the concentration of DNA in a sample by performing a non-specific PCR using a partially degenerate primer. This technique has been demonstrated successfully using DNA from two important pathogens, *Staphylococcus aureus* and *Vibrio cholerae*. After employing this method a small amount of newly synthesized DNA can be used for subsequent amplification reactions with specific primers. By adding this preliminary step the amount of DNA available is increased so that many more PCRs can be performed with the initial DNA sample than originally possible. Additionally, while the concentration of DNA is increased, the concentration of contaminants (such as hemoglobin) that inhibit PCR is not, therefore allowing for the dilution of the sample while maintaining an adequate concentration of DNA for analysis. This method can also increase the sensitivity of DNA detection with specific primer pairs by increasing the concentration of the target DNA. This can be done by using an initial amplification step with the primer 6-MW (ref) and a 37°C annealing temperature. These partially degenerate primers are then removed by column filtration and a small aliquot of this DNA is added to subsequent amplification reactions using specific primers. To date we have tested 23 primer pairs for *Staphylococcus aureus* and 18 primer pairs for *Vibrio cholerae*. Using this method we were able to detect as little as 10 fg of target DNA for many of the primer pairs developed to identify these organisms. By adding this preliminary step we observed an increase in sensitivity of up to 1000-fold, with one primer pair for *Vibrio cholerae* showing a 10,000-fold increase in sensitivity.

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